

BIOLOGY 163 LABORATORY

USE OF THE MICROPIPETTE

(Revised Fall 2007)

Digital micropipettes are used to transfer small volumes of solutions from one place to another. Each micropipette has a specific volume range. These pipettes are expensive, precision instruments and must be used with care. Their accuracy is dependent upon their proper use and reliable pipette tips. Tips of different manufacturers can be a source of variation in micropipetting. BEWARE!

Please remember these four DON'TS when using a digital micropipette:

Don't rotate the volume adjuster beyond the upper or lower range of the pipette.

Don't use a pipette without a tip in place. Doing so could ruin the precision piston that measures the volume of fluid.

Don't lay down or invert a pipette that has a filled tip. Fluid could run back into the piston.

Don't let the plunger snap back after withdrawing or ejecting fluid. Doing so could damage the piston.

Specific Instructions for Using a Micropipette

Several brands of micropipettes exist. Although different brands may vary somewhat in construction and specific features, the basic operation of all micropipettes is similar. Read the following instructions carefully before using a micropipette. After you have done so, practice operating the pipette dry (without a sample), and then with a bit of distilled water. Once you are comfortable with basic micropipette operation, complete the practice exercises on the next page.

1. Select a pipette that has a volume range that includes the volume you will use. **DO NOT USE PIPETTES FOR VOLUMES OUTSIDE OF THEIR INTENDED RANGE!**
2. Firmly press a new tip onto the pipette. Do not touch the tip with your hands.
3. Adjust the pipette to the desired volume within the range of the pipette. (Be sure to locate the decimal point (a line) properly when reading the volume setting!) Some models are adjusted using the plunger button (which may or may not have a locking mechanism). Other are adjusted with a separate knob or wheel. Ask your instructor if you need help with a particular micropipette.
4. To draw liquid into the micropipette tip:
 - a. Depress the control button to the **FIRST STOP** (there are three) and hold in that position.
 - b. Holding the micropipette nearly vertical, immerse the tip 1-3 mm into the liquid to be moved.
 - c. Draw the fluid into the tip by **SLOWLY** letting the control button slide back. Wait 1-2 seconds to be sure that the full volume of sample is drawn into the tip.
 - d. Slip the filled tip out, moving it along the inside of the vessel to dislodge excess liquid adhering to the outside of the tip. Before proceeding, check to be sure that liquid fills the tip and that no air is at the end of the tip. To avoid pipetting errors, learn to recognize the approximate level to which particular volumes fill the tip. *If air is noted in the tip during intake, dispense the sample back into the reagent vessel, and repeat more slowly and evenly. If air is noted a second time, discard the tip and use a new one.*

5. To dispense the liquid:
 - a. Touch the tip near the bottom of the inside of the receptacle (e.g., microcentrifuge tube or agarose gel).
 - b. Slowly depress the control button to the **FIRST STOP** and wait 1-3 seconds; most of the liquid will be expelled.
 - c. Press the control button to the **SECOND STOP** to blow out any residual liquid in the tip.
 - d. To remove the pipette from the dispensing location, keep the button depressed and slide the tip along the inside of the vessel or the gel well. This prevents sucking liquid back into the tip.
 - e. Eject the tip into a tip disposal container by pressing the control button to the **THIRD STOP** or pressing the separate tip eject button (depending on the brand of micropipette you are using).
 - f. Attach a new tip and proceed, or store the micropipette.

PRACTICE EXERCISES

Simulated reaction mixture

- a. Label three 1.5 ml microcentrifuge tubes A, B, and C with a permanent marker.
- b. Using the appropriate micropipette(s), make three mixtures as indicated in the following matrix. Use a new tip for each different reagent. If a tip comes in contact with a previously pipetted solution, discard it and use a new tip.

TUBE	REAGENT		
	Yellow	Green	Red
A	8 μ l	5 μ l	5 μ l
B	8 μ l	6 μ l	4 μ l
C	8 μ l	7 μ l	3 μ l

- c. A total of 18 μ l was added to each tube. To check your accuracy, set an appropriate pipette to 18 μ l and carefully withdraw the solution from each tube:
 - If the tip is just filled leaving no liquid in the tube, did you pipette accurately?
 - If fluid is left in the tube when the tip is filled, did you pipette accurately?
 - If an air space is left in the tip end leaving no liquid in the tube, did you pipette accurately?
- d. Repeat steps a-c until you are able to pipette with consistent accuracy.

Testing your technique

Using a milligram (0.001g) balance with a draft shield, test your micropipetting technique by weighing out aliquots of distilled water. You should try several different volumes (within the range of the micropipette you are using), and also try several samples of the same volume.

(HELPFUL TIP: Because 1 μ l of water weighs 1mg, you should be able to easily calculate how much each sample should weigh!)

- Did you micropipette accurately throughout the range of the micropipette?
- Did you micropipette consistently? (i.e., Were your results repeatable?)